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# CORTICAL SPREADING DEPRESSION INDUCES OXIDATIVE STRESS IN THE TRIGEMINAL NOCICEPTIVE SYSTEM

A. SHATILLO,[a†] K. KOROLEVA,[b†] R. GINIATULLINA,[a] N. NAUMENKO,[a] A. A. SLASTNIKOVA,[c] R. R. ALIEV,[c,d] G. BART,[a] M. ATALAY,[e,f] C. GU,[g] R. KHAZIPOV,[b] B. DAVLETOV,[g] O. GROHN[a] AND R. GINIATULLIN[a*]

[a] *Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland*

[b] *Laboratory of Neurobiology, Kazan Federal University, Kazan, Russia*

[c] *Moscow Institute of Physics and Technology, Dolgoprudny, Russia*

[d] *Institute of Theoretical and Experimental Biophysics, RAS, Puschino, Russia*

[e] *Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland*

[f] *Institute of Physiology, University of Eastern Finland, Kuopio, Finland*

[g] *MRC Laboratory of Molecular Biology, Cambridge, UK*

**Abstract—Indirect evidence suggests the increased production of reactive oxygen species (ROS) in migraine pathophysiology. In the current study we measured lipid peroxidation product in the rat cortex, trigeminal ganglia and meninges after the induction of cortical spreading depression (CSD), a phenomenon known to be associated with migraine aura, and tested nociceptive firing triggered by ROS in trigeminal nerves *ex vivo*. Application of KCl to dura mater in anesthetized rats induced several waves of CSD recorded by an extracellular electrode in the cortex. Following CSD, samples of cortex (affected regions were identified with blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI)), meninges from left and right hemispheres and trigeminal ganglia were taken for biochemical analysis. We found that CSD increased the level of the lipid peroxidation product malondialdehyde (MDA) in the ipsilateral cerebral cortex and meninges, but also in both ipsi- and contralateral trigeminal ganglia. In order to test the pro-nociceptive action of ROS, we applied the mild oxidant hydrogen peroxide to isolated rat hemiskull preparations including preserved trigeminal innervations. Application of hydrogen peroxide to meninges transiently enhanced electrical spiking activity of trigeminal nerves showing a pro-nociceptive action of ROS. In the presence of hydrogen peroxide trigeminal nerves still responded to capsaicin by burst of spiking activity indicating integrity of neuronal structures. The action of hydrogen peroxide was mediated by TRPA1 receptors as it was abolished by the specific TRPA1 antagonist TCS-5861528. Using dorsal root ganglion sensory neurons as test system we found that hydrogen peroxide promoted the release of the migraine mediator calcitonin gene-related peptide (CGRP), which we previously identified as a trigger of delayed sensitization of trigeminal neurons. Our data suggest that, after CSD, oxidative stress spreads downstream within the trigeminal nociceptive system and could be involved in the coupling of CSD with the activation of trigeminovascular system in migraine pathology.** 

**Key words: trigeminal neurons, trigeminal ganglion, CSD, ROS, CGRP, migraine.**

## INTRODUCTION

Migraine is a common complex neurological disease associated with neuronal hyperexcitability, neuroinflammation in meninges and release of multiple active substances known as 'migraine mediators' (Pietrobon and Striessnig, 2003; Ho et al., 2010). One third of migraine cases are associated with aura (Silberstein and Lipton, 1993; Olesen et al., 2009), a phenomenon linked to cortical spreading depression (CSD, Leao, 1944). The link between CSD and migraine aura was initially suggested by Milner (1958) and later directly demonstrated using functional magnetic resonance imaging (fMRI) in a patient during a migraine attack (Hadjikhani et al., 2001). CSD represents a strong wave of neuronal depolarization associated with the activation of glial cells and blood vessels, which propagates across the gray matter (Somjen et al., 1992; Somjen, 2001; Charles and Brennan, 2009). One issue which is central but largely unclear is the coupling of CSD with the activation of the trigeminovascular system (Olesen et al., 2009). Although the induction of CSD in rats was shown to be followed by delayed and persistent activation of trigeminal neurons (Zhang et al., 2010, 2011), the mechanism of this phenomenon remains elusive.

Dramatic, although transient, metabolic changes in the cerebral cortex associated with intracellular calcium overload during CSD (Takano et al., 2007), could be potential inducers of oxidative stress (Viggiano et al., 2011). Diffusible reactive oxygen species (ROS) generated during oxidative stress can, potentially, activate nociceptive signaling via redox-sensitive ion channels. Therefore, we hypothesized, that diffusible ROS

*Corresponding author. Tel: +358-403553665; fax: +358-17163030.
E-mail address: rashid.giniatullin@uef.fi (R. Giniatullin).
† These authors contributed equally to this work.
*Abbreviations:* ANOVA, analysis of variance; BOLD, blood oxygen level-dependent; CGRP, calcitonin gene-related peptide; CSD, cortical spreading depression; DRG, dorsal root ganglion; fMRI, functional magnetic resonance imaging; LFP, local field potential; MDA, malondialdehyde; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances.

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